Western Spruce Budworm and Development
as a Function of Mount St. Helen's Ash
in Artificial Versus Natural Diets.

Cooperative agreement

Supplement 80-105-CA
Univ. Mont. 9/8/80 - 9/30/82

Western Spruce Budworm Mortality and Development as a Function of Mount St. Helen's Ash in Artificial versus Natural Diets

Jerry J. Bromenshenk, R.C. Postle, G.M. Yamasaki, D.G. Fellin, and H.E. Reinhardt

Jerry J. Bromenshenk
Departments of Zoology and Botany
University of Montana
Missoula, Montana 59812

R.C. Postle
Reclamation Science
Montana State University
Bozeman, Montana 59717

Gayle M. Yamasaki
David G. Fellin
Forestry Sciences Laboratory
USDA Forest Service
Missoula, Montana 59806

H.E. Reinhardt
College of Arts and Sciences
University of Montana
Missoula, Montana 59812

Coop - INT station
U of MT

ABSTRACT

Ingestion of volcanic ash from Mount St. Helen's by larvae of the western spruce budworm, *Choristoneura occidentalis (Lepidoptera: Tortricidae),* significantly altered rates of development, survival and mortality, and parasitism. The observed effects differed depending upon the method of administration of the ash. Second through sixth instar larvae fed ash mixed into an artificial diet displayed significantly increased rates of development but no significant changes in survival or mortality even when the diet contained 50 percent ash. Fourth through sixth instar larvae fed ash-dusted foliage showed significantly increased mortality at all but the lowest deposition amounts tested. In addition, ash levels that affected budworm survival significantly reduced the numbers of larvae killed by parasites. Ingestion of large amounts of ash tended to reduce pupal and adult size, particularly of males, and in some cases induced dwarfing of adults.

Overwintering second instar larvae were obtained from the Deer Lodge National Forest near Pipestone, Montana, April, 1981. One hundred bolts were cut from seven Douglas Firs, transported to Missoula, Montana, and placed in a walk-in cooler ($5^{o}C$). Bolts brought to room temperature induced emergence; mean emergence rate per tree ranged from 238.11 to 1,267.66 larvae/m^2 of bark surface (1) for a total of 15,072 larvae from seven trees.

Ash utilized in our tests was acquired in October, 1981 from piles of ash found at St. Maries, Idaho. The top several centimeters of ash were discarded because of visible weathering. Ash that fell on Idaho, eastern Washington, and western Montana was finer in texture than that which fell near Mt. St. Helens, being abrasive with particle sizes ranging from less than 2 microns (2 to 6 percent) to 50 microns (22 to 88 percent by weight) (2,3). The ash contained small amounts of toxic elements such as arsenic, mercury, lead, cadmium, and fluoride (4,5).

Treatment Application

Graded dosages of ash were administered in order to determine toxicity and to examine possible differences on effects resultant from ingestion of ash contained in an artificial diet or on foliage.

In order to apply dust to foliage in controlled amounts, we utilized a vacuumchamber (6,7). Foliage specimens to be dusted were placed in the bottom of bell-jar shaped chambers while a weighed amount of ash was suspended on a watch glass in the top. Chambers were evacuated to 63.5 cm of mercury, then an electrically controlled solenoid valve was opened to admit outside air, which imploded, dispersing ash. With this apparatus, we were able to simultaneously dust two sets of twelve branchlets at the same

or different dosages. These chambers applied a uniform coating of dust to all surfaces of needles and stems, with just slightly more dust on the adaxial surfaces. This is consistent with observations of ash on needles deposited by the volcanic eruptions. The amount of ash in micrograms per centimeter squared versus amount delivered (gms of ash) was determined using filter paper targets (Figure 1). Based on this information, we chose eight independent dusting levels ranging from 0 to 32. The upper limit corresponds to the holding capacity of foliage for ash.

For administering ash in an artificial food, the volcanic material was mixed with water to make a slurry before being added to the contents of the diet (8). We tested seven concentrations of ash ranging from 0 to 50% replacement of the dry ingredients (w/w). This exceeded the uppermost range of values for ash per gram of foliage measured on vegetation collected from areas subjected to deposition from the volcano (9).

Rearing and Determination of Dose Effects

Emergent larvae were reared on artificial diet until dosed (8). For the foliage tests, terminal branchlets (15 to 25 cm in length) were taken from mature Douglas Firs. Freshly cut foliage was dusted and placed in a Randall cage, the stem immersed in water. Fourth instar larvae were selected at random and put on the branchlets. Experiments were set up in eight replicate blocks of 80 cages with five larvae per cage, eight dosage levels (0, 2, 4, 8, 12, 16, 24, and 32 gms), ten cages per dosage. Cages in each block were randomly distributed on racks in a greenhouse. Temperature, light, and humidity reflected natural diurnal fluctuations. Humidity ranged from 16 to 96 percent and temperature from 11.1 to 34.4^{o} C. Counts were conducted weekly to determine for each cage percent living, percent dead, and percent killed by parasites.

For the artificial diet tests, larvae were reared individually in disposable 5 cm tissue culture dishes containing diet media which was replaced as needed. Three independent experimental runs were carried out, each consisting of 200 budworm larvae subjected to four treatment levels of ash. Run 1 treatment levels were 0, 8, 17.5, and 25 percent ash (w/w). Run 2 and 3 levels were 0, 30, 40, and 50 percent. Rearing was conducted in a windowless laboratory room. Temperature and humidity were not strictly controlled, although forced air cooling was utilized to keep maximum temperatures below 25^{o} C. Daily determinations included stage of development (second, third, fourth, or fifth instar larvae, pupa, adult) and whether each budworm was alive or dead, and whether the insect was parasitized. Budworm surviving to the pupal stage were sexed and weighed.

Statistics

For dusted foliage, the hypotheses tested were whether different levels of ash have any effect on either the survival or the mortality of western spruce budworm. The model utilized was:

$$Y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \Sigma_{ijk} \qquad (10,11)$$

Y = % of spruce budworm living, dead, or parasitized
μ = overall mean
α_i = replication
β_j = treatment (levels of ash)
$\alpha\beta_{ij}$ = interaction between replication and treatment
Σ_{ijk} = error term

The ANOVA table took the form:

Source	df	EMS
Rep	$(r-1)$	$\sigma^2 + tn\Sigma\alpha_i/(r-1)$
Trt	$(t-1)$	$\sigma^2 + rn\Sigma\beta_j/(t-1)$
Rep*Trt	$(r-1)(t-1)$	$\sigma^2 + n\Sigma\Sigma(\alpha\beta)_{ij}/(r-1)(t-1)$
Error	$rt(n-1)$	σ^2
Total	$(rtn-1)$	

Before any of the analyses were run, the methods and data were checked to make certain they met all the assumptions of linearity, additivity, independence, and homogeneity of variance (Bartlett's test). The results of analysis of variance were evaluated by using either Tukey's Multiple Range Test, 95% Confidence Interval, or both, plus plotting the results.

For ash in artificial diet, the hypotheses tested, models utilized, and assumptions were essentially the same as for the tests of dusted foliage. In addition, we tested whether the ash had any effect on development rate. To help get a measure of the effect of time going from instar to instar for different levels of ash, the following linear regression analyses were run:

$$Y_{ij} = b_o + b_1 X_i + \varepsilon_{ij} \qquad (12,13)$$

Y_1 = instar
b_o = intercept
X_1 = time
b_1 = coefficient for independent variable (slope)

To determine whether there were differences between the mean number of days needed for transition to each instar, the following analyses of variance were run:

$$Y_{ijk} = \mu + \beta_i + \varepsilon_{ij} \qquad (10,11)$$

Y_{ikj} = day
μ = overall mean of the model
β_i = ash levels or Trt level (0,16,35,50)
ε_{ij} = overall error term

Source	df	EMS
Trt	t-1	$\sigma_\varepsilon^2 + n\Sigma\alpha_i/(t-1)$
Error	t(n-1)	σ_ε^2
Total	(tn-1)	

To get a measure of the effect of this difference the following linear regression was run:

$$Y = X^b \qquad (12,13)$$

Y = number of days
X = instar (3-pupa)

Results

Feeding trials with ash-dusted foliage are summarized in Figure 2 and Tables 1 and 2. Survival diminished significantly with time and with increased exposure to ash. Application rates of 8 grams or more of ash to foliage produced significant reductions in survival compared to the controls. Only a slight interaction was detected between observation time and dose level. Ash application rates in excess of 24 grams significantly lowered the percent larvae killed by parasitoids as compared to the 0 and 4 gram treatments (Table 3). Percent mortality, excluding death for parasitoids, demonstrated essentially the same results as percent survival. Mean percent mortality attributed to ash ranged from 6 to 18 percent for each stage of development, whereas that from parasitoids ranged from 16 to 30 percent.

The results of tests of ingestion of ash mixed into artificial diet were different from those for ash-dusted foliage. We could not detect dose-response differences with respect to ash induced alterations of survival or mortality, although in tests of 0 to 25 percent ash (w/w), survival of larval instars appeared to be better for the controls (Figure 3a.). A protozoan (*Nosema bombycis*) killed a considerable proportion of the larvae. For later trials with higher dosages of ash (0 to 50 percent), the diet was treated with a prophylactic medication (14). Suppression of the *Nosema* population removed the apparent "effect" observed in the earlier tests; percent survival was virtually the same for controls as for dosed budworm (Figure 3b.). Ash in the diet media significantly affected development rate by shortening the duration of the pupal stage for most ash treatments compared to the controls (Figures 4a. and 4b., Table 4).

Occasionally, adults of budworm reared on ash-laden foliage were dwarfed, some being less than one-half normal size. Therefore, for the ash in diet experiments, we weighed surviving pupae. Mean weights for males

tended to decline with increasing exposure levels to ash, ranging from means of 0.100 to 0.105 gm for 0% ash to lows of 0.67 to 0.68 gm for 30% and 40% ash, respectively. For females, we could not detect weight changes for some trials, the mean weights ranged from 0.103 to 0.107 gms across the dose levels. In one replication, a weight decline did occur, ranging from a mean of 0.114 gm for 0% ash to 0.074 gm for 50% ash.

Before feeding ash to budworm, we carried out some preliminary tests of the response fo budworm larvae to dermal contact with ash. Using our vacuum chamber, we applied thin coatings of ash to petri dishes. Third instar larvae placed on clean glass for the most part avoided contact with ash, turning away whenever encountering the material. Individuals placed in contact with the ash usually began immediately to crawl about. Insects reaching areas of clean glass seldom returned to the ash; those failing to escape the ash generally perished. Often, larval movements in the ash ceased within a matter of minutes.

Discussion

Ash on foliage caused significant budworm mortality. The heaviest ash application approximated the amount found adhering to field-collected foliage five months after the May eruptions. At this level, our tests indicate that ash could be expected to reduce field populations by 15 to 20 percent. It may reduce the amount of insect caused foliage damage by an even greater amount. We noticed that budworm consumed less foliage with increasing amounts of ash as evidenced by frequency of replenishing consumed branchlets in the Randall cages. These findings are similar to those for experiments with adult root weevils, *Otiorhynchus ovatus* (L.) and *O. sulcatus* (F.). Adult weevils demonstrated inhibition of feeding and mortality when provided strawberry leaves coated with ash (5 leaves, 1 weevil per treatment). The effect was more pronounced with wet leaves versus dry leaves dipped in ash (15) probably because the ash tends to clump in heavy deposits on wet surfaces.

Our foliage treatment results indicate that parasites of budworm may be impacted to a greater degree than budworm. Based on the decline of percent mortality attributable to parasitoids, effectiveness of these organisms of reducing budworm numbers could be reduced by volcanic ash by more than 50 percent.

Our findings of (a) a lack of mortality effects, (b) shortened development periods, and (c) somewhat reduced male pupal weight for budworm fed ash mixed into a diet media differ from other published results. Tests of an herbivore *Manduca sexta* force fed artificial diets containing 5 percent w/w of water substituted by a variety of intert materials, including volcanic ash did not demonstrate inhibition of larval development with respect to larval weight, duration of penultimate instar, or days required to pupate (n = 6 for each

treatment) (16). However, in our budworm tests we used a broader range of dosages, greenhouse rearing rather than controlled environment chambers, considerably more replications, and longer exposure periods. As with our foliage testing, it appeared that the ash in diet media inhibited parasitoids and was about as effective in controlling these agents as the prophylactic drug treatment.

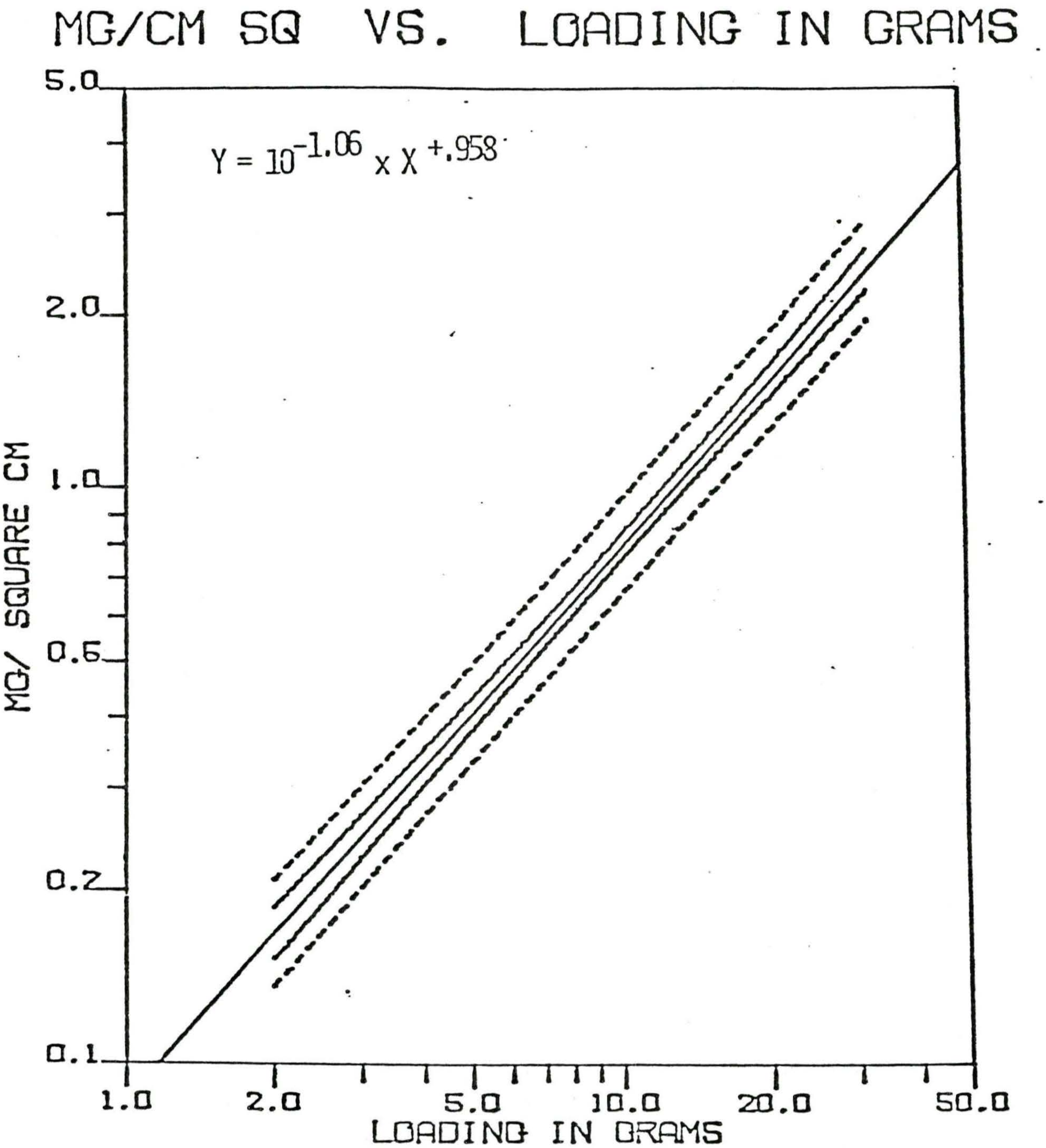
Different results for ash administered on foliage versus artificial diets were not simply a matter of dose delivered. The maximum application rate of dust to foliage was equivalent to approximately 40 percent ash to dry weight of needles, comparable to the 50 percent upper limit for ash mixed into the artificial diet. Artificial diet results suggest that chemically the ash is relatively non-toxic, while the foliage results suggest that abrasion from loosely deposited ash on the needle surface may be a major factor in mortality. Also, mixing the ash into diet media may modify physical and chemical properties. Increased development rate suggests that chemicals in ash could have some nutritive benefit or stimulate digestion, but this seems unlikely given the dissimilar results for foliage. Alternatively, ash may change the physiological processes and biochemistry of the foliage in a manner not reproducible in the artificial diet. Occasional dwarf adults and some reduction of pupal weight as well as increased development rate suggest some type of stress response, possibly starvation. Feeding experiments with the large aspen tortrix *Choristoneura conflictana* have clearly demonstrated effects of starvation and of five different host plants on larval survival and pupal weight. Larvae fed alder took over twice as long to develop as those fed aspen (17). These effects in many ways were similar to those we observed with volcanic ash.

Whatever the mechanisms involved, our investigations demonstrate the potential for error in assessing the effects of factors affecting insect

population dynamics based on the results of a single type of treatment or on very limited sample sizes. Many laboratories routinely conduct toxicological bioassays of chemical insecticidal formulations using laboratory populations, reared in controlled environment chambers, on artificial diets. A recent warning appeared concerning recommending chemicals for field-testing based on a single type of bioassay (18). Our data indicates that artificial as compared to "more natural" foliage diets can produce drastically different results. Also, suppression or elimination of parasitoids in our tests, such as normally occurs when utilizing laboratory colonies, would have prevented us from discovering the effects of ash on the host-parasitoid relationship.

Based on the combined results of our tests, we conclude that the May 18, 1980 eruption probably had a substantial impact on budworm populations since the early instars would have been subjected to direct contact with ash, being particularly vulnerable to entrapment, abrasion, and dessication. Larvae surviving the intial ash deposition should have sustained additional losses during the period of their feeding on foliage. Unfortunately, at the same time, the effectiveness of parasitoids as a factor in budworm mortality probably was ~~somewhat~~ diminished.

FIGURE 1



MG/CM SQ VS. LOADING IN GRAMS
Y = 10^-1.06 x X^+.958
5.0
2.0
1.0
0.6
0.2
0.1
MG/ SQUARE CM
1.0
2.0
5.0
10.0
20.0
50.0
LOADING IN GRAMS

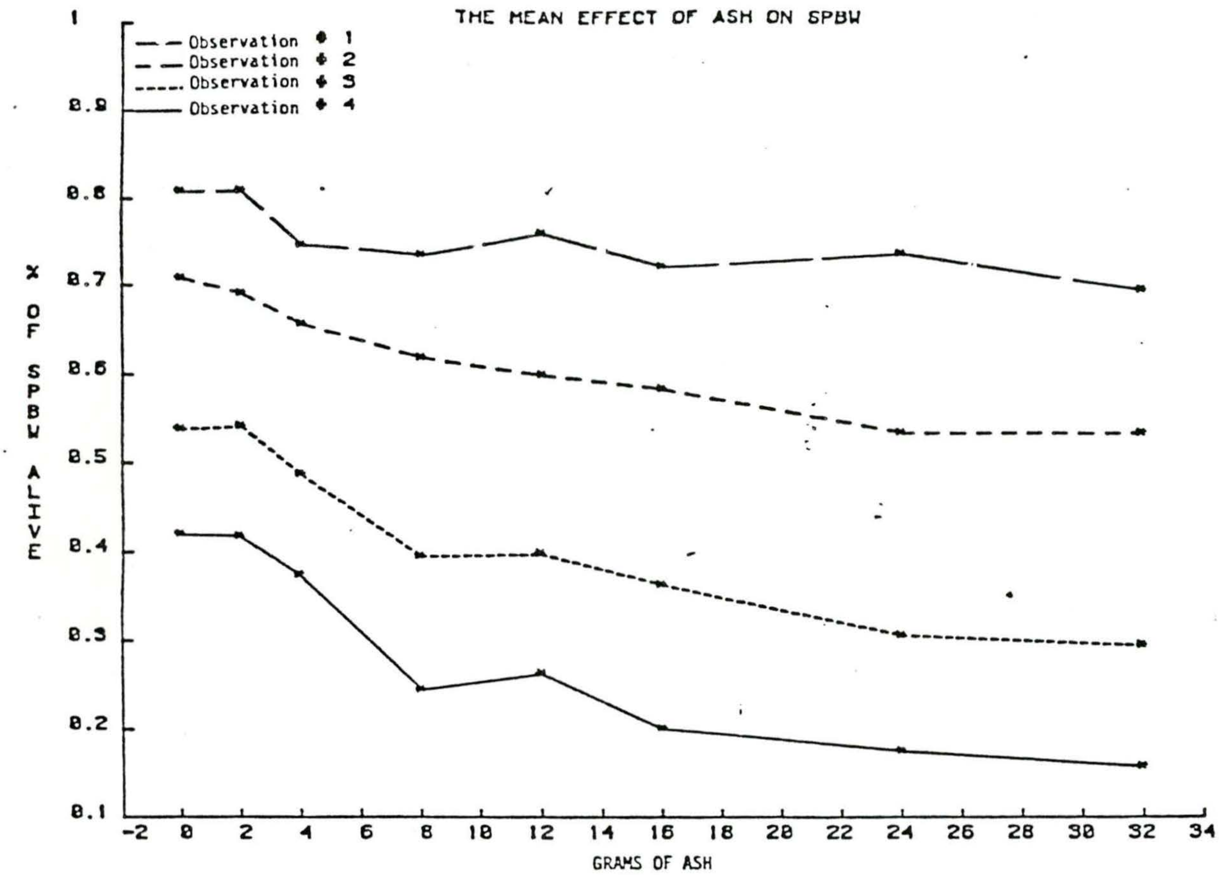


FIGURE 2. The Mean Effect on Spruce Budworm Larvae of Ingesting Ash-Dusted Foliage. The observation periods cover the time from the original count (t=1) until the completion ot the experiment when the Randall cages were disassembled and carefully searched for living larvae (t=4).

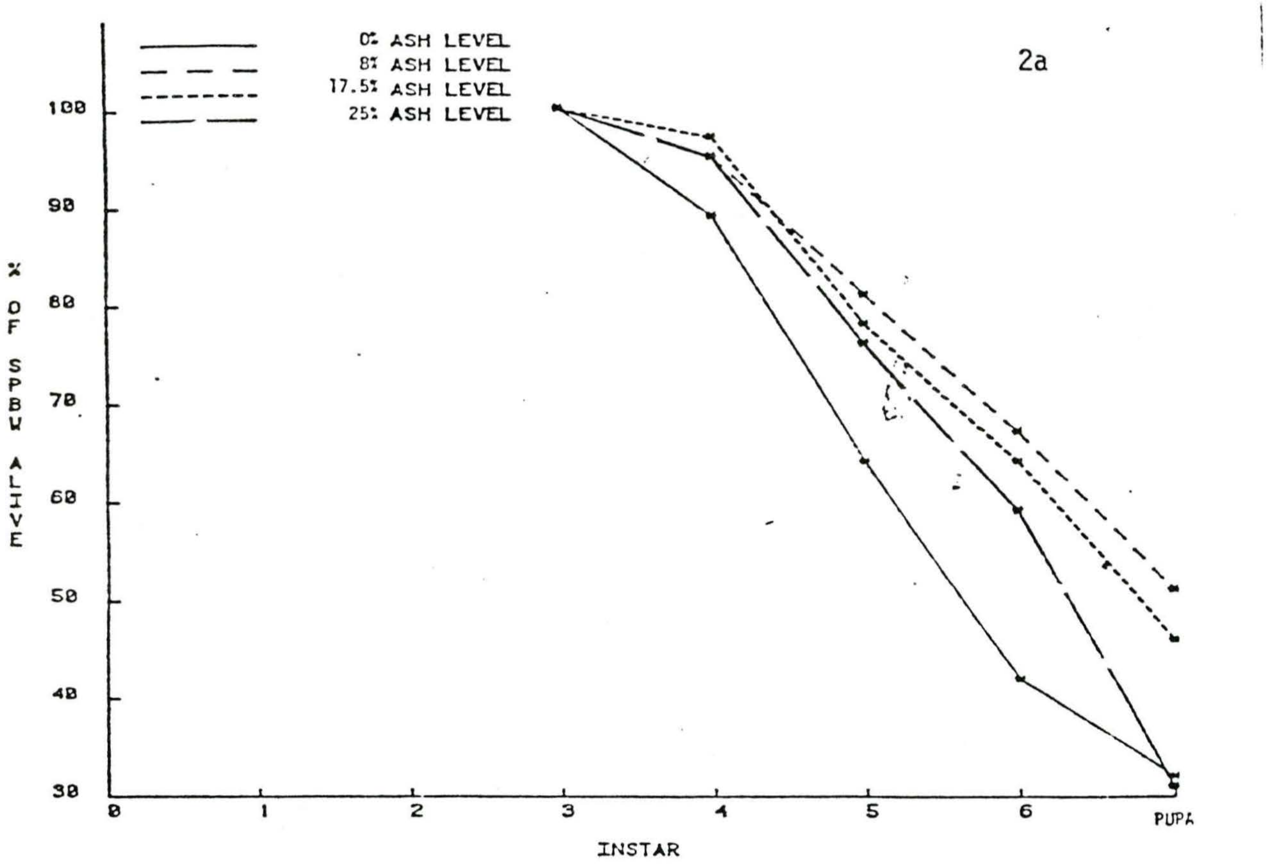


Figure 3a. Mean Survival of Budworm Exposed to Volcanic Ash in an Artificial Diet. All populations were infected with *Nosema spp.*

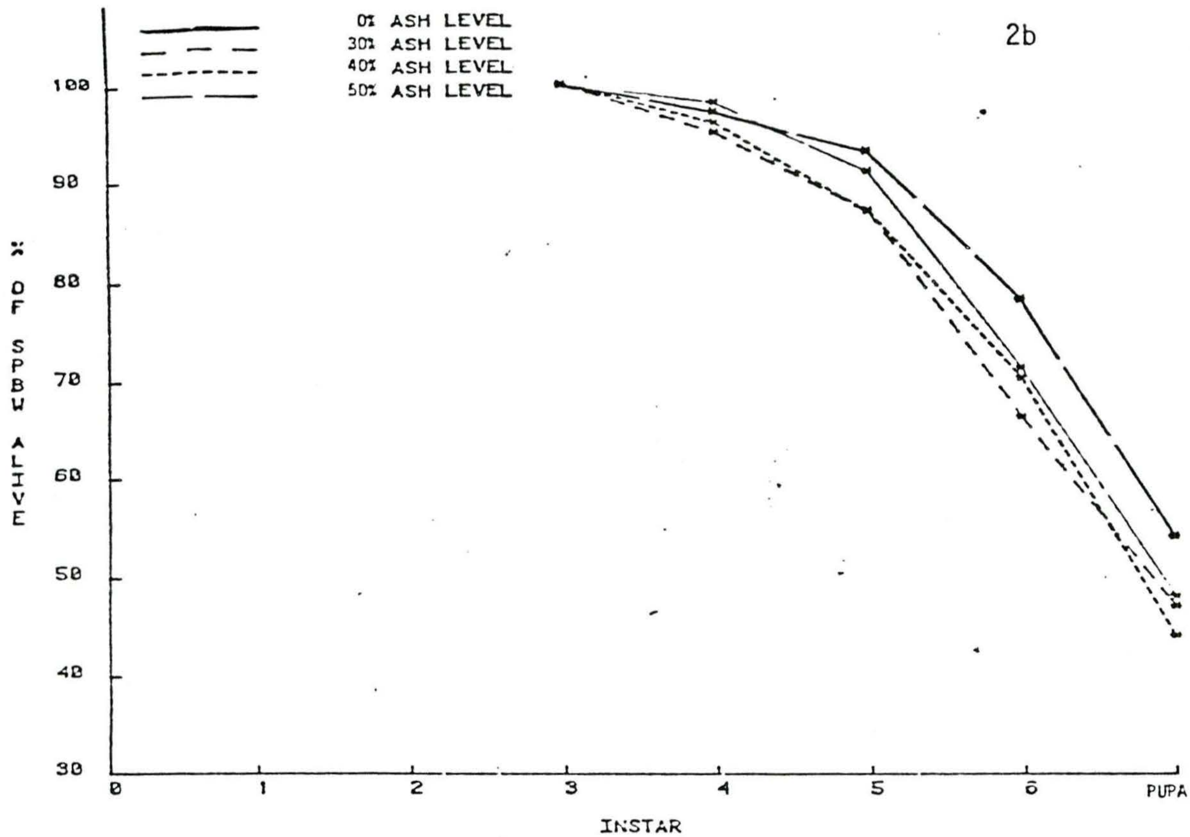
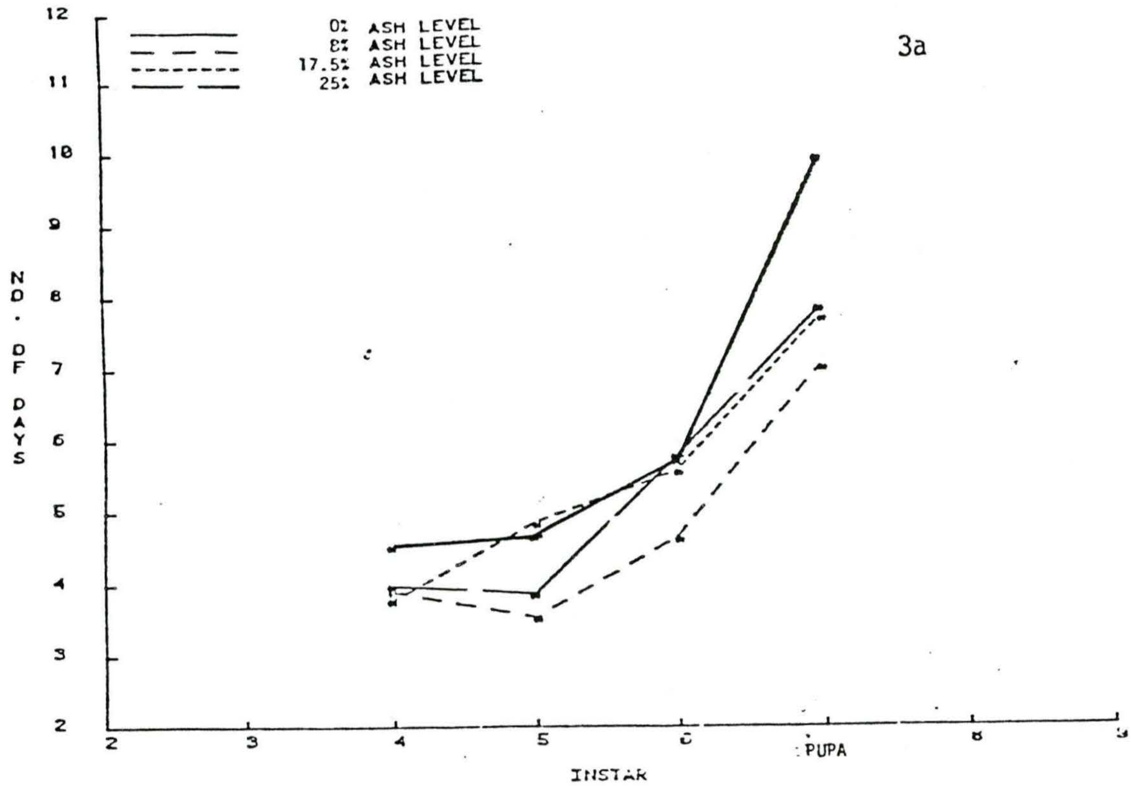


Figure 3b. Dose Trials of High Treatment Levels of Ash and Protozoan Population Supressed by Prophylactic Treatment.



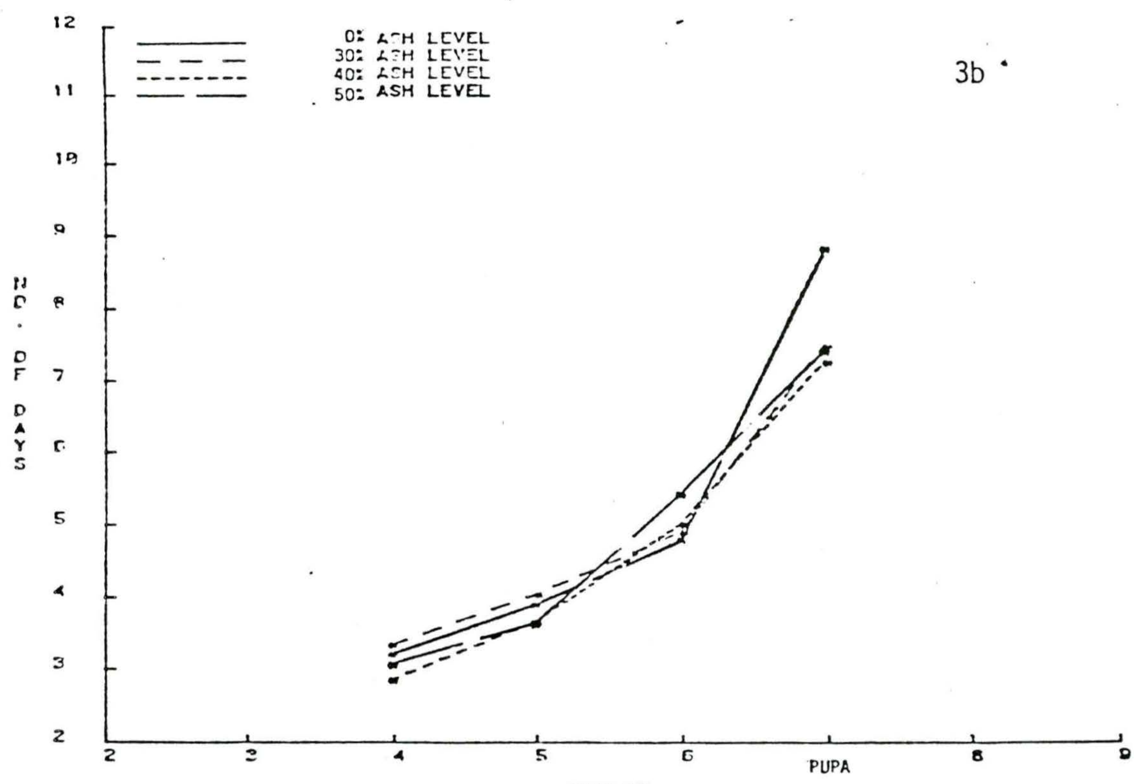


Figure 4 a,b. Mean Development Periods for Budworm Ingesting Ash in a Diet Medium

Table 1

ANOVA for Trials of Ingestion of Foliage Dusted with Volcanic Ash and Basic Statistics for % Alive

Source	df	SS	MS	F Ratio	F Prob.
Treatment	31	99.07576	3.19599	65.3381	0.000
Ash	7	12.7666	1.8238	37.285	0.000
Time	3	84.4404	28.14682	575.43	0.000
Ash x Time	21	1.8687	0.088986	1.8192	0.004
Error	2528	123.6564	0.0489		
Total	2559	222.7322			

% Ash	N	Mean % Alive	Std	95% Confidence Interval
0	320	0.6181	0.2608	0.5894 $\leq$ 0.6181 $\leq$ 0.6468
2	320	0.6138	0.2646	0.5847 $\leq$ 0.6138 $\leq$ 0.6429
4	320	0.5563	0.2726	0.5263 $\leq$ 0.5563 $\leq$ 0.5863
8	324	0.4975	0.2912	0.4656 $\leq$ 0.4975 $\leq$ 0.5294
12	320	0.5038	0.2945	0.4714 $\leq$ 0.5038 $\leq$ 0.5362
16	320	0.4663	0.3007	0.4332 $\leq$ 0.4663 $\leq$ 0.4994
24	320	0.4375	0.3109	0.4033 $\leq$ 0.4375 $\leq$ 0.4717
32	316	0.4196	0.2956	0.3868 $\leq$ 0.4196 $\leq$ 0.4524
Overall Mean	2560	0.5142	0.2950	0.5028 $\leq$ 0.5142 $\leq$ 0.5256

Table 2

ANOVA for Trials of Ingestion of Foliage Dusted with Volcanic Ash and Basic Statistics (% Dead)

Source	df	SS	MS	F Ratio	F Prob.
Treatment	31	32.03632	1.0334296	34.64	0.000
Ash	7	6.50888	0.9298404	31.166	0.000
Time	3	21.65912	7.219706	241.99	0.000
Ash x Time	21	3.86831	0.184205	6.174	0.000
Error	2528	75.42242	0.02983		
Total	2559	107.45870			

% Ash	N	Mean % Dead.	Std	95% Confidence Interval
0	320	0.0619	0.1208	$0.0486 \leq 0.0619 \leq 0.0752$
2	320	0.0600	0.1243	$0.0463 \leq 0.0600 \leq 0.0737$
4	320	0.0794	0.1215	$0.0634 \leq 0.0794 \leq 0.0954$
8	324	0.1475	0.1974	$0.1259 \leq 0.1475 \leq 0.1691$
12	320	0.1450	0.2003	$0.1229 \leq 0.1450 \leq 0.1671$
16	320	0.1825	0.2528	$0.1547 \leq 0.1825 \leq 0.2103$
24	320	0.1794	0.2488	$0.1520 \leq 0.1794 \leq 0.2068$
32	316	0.1804	0.2464	$0.1531 \leq 0.1804 \leq 0.2077$
Overall Mean	2560	0.1295	0.2049	$0.1216 \leq 0.1295 \leq 0.1374$

Table 3

ANOVA for Rates of Parasitism on Larvae Feeding on Foliage Dusted with Volcanic Ash and Basic Statistics

Source	df	SS	MS	F Ratio	F Prob.
Treatment	7	1.800	0.257	5.220	0.00
Error	632	31.128	0.043		
Total	639	32.928			

% Ash	N	Mean % Parasitized	Std	95% Confidence Interval
0	80	0.2975	0.0466	0.2871 $\leq$ 0.2975 $\leq$ 0.3079
2	80	0.2750	0.0500	0.2639 $\leq$ 0.2750 $\leq$ 0.2861
4	80	0.2950	0.0516	0.2835 $\leq$ 0.2950 $\leq$ 0.3065
8	81	0.2741	0.0564	0.2616 $\leq$ 0.2741 $\leq$ 0.2866
12	80	0.2525	0.0483	0.2418 $\leq$ 0.2525 $\leq$ 0.2632
16	80	0.1775	0.0446	0.1676 $\leq$ 0.1775 $\leq$ 0.1874
24	80	0.1825	0.0539	0.1705 $\leq$ 0.1825 $\leq$ 0.1945
32	79	0.1595	0.0424	0.1500 $\leq$ 0.1595 $\leq$ 0.1690
Overall Mean	640	0.2394	0.515	0.2354 $\leq$ 0.2394 $\leq$ 0.2434

Table 4

Rate of Pupal Development for Spruce Budworm

Fed Ash in an Artificial Diet Medium

% Ash Level	N	Mean	Std	95% Confidence Interval
0	94	8.8830	3.8181	$8.1009 \leq 8.8830 \leq 0.6651$
0	30	9.9232	3.9994	$8.4939 \leq 9.934 \leq 11.3732$
8	48	7.0417	1.9347	$6.4921 \leq 7.0417 \leq 7.5913$
17.5	46	7.7174	2.6303	$6.9564 \leq 7.72 \leq 8.4836$
25	29	7.8276	2.8917	$6.7716 \leq 7.83 \leq 8.8884$
30	82	7.5488	2.5587	$6.9868 \leq 7.5488 \leq 8.1108$
40	80	7.3250	2.7640	$6.7109 \leq 7.3250 \leq 7.9390$
50	100	7.4600	2.9074	$6.8832 \leq 7.4600 \leq 8.0368$

References and Notes

1. L. Theroux, Compliation of Emergence records for U.S.F.S., Region 1, Insect and Disease Division.

2. J.S. Fruchter, D.E. Robertson, J.C. Evans, K.B. Olsen, E.A. Lepel, J.C. Laul, K.H. Abel. R.W. Sanders, P.O. Jackson, N.S. Wogman, R.W. Perkins, H.H. Van Tuyl, R.H. Beauchamp, J.W. Shade, J.L. Daniel, R.L. Erikson, G.A. Sehmel, R.N. Lee, A.V. Robinson, O.R. Moss, J.K. Briant, W.C. Cannon. Science 209,1116 (1980).

3. W.S. Moen and G.B. Lucas. Washington Dept. Natural Resources, Report of Investigations 24, 13 (1981).

4. J.S. Fouchter, et al., op. cit. 209, 1116 (1980).

5. W.S. Moem and G.B. Lucas, op. cit., 24, 13 (1981).

6. E.L. Atkins, L.D. Anderson, T.O. Tuft. J. Econ. Entomol. 47,965 (1954).

7. E.L. Atkins, E.A. Greenwood, R.L. MacDonald. University of California Division of Agricultural Science Leaflet 2287, 3 (1975).

8. R.L. Lyan, S.J. Brown, J.L. Robertson. J. Econ. Entomol. 65, 928 (1972).

9. D.E. Bildeback. AAAS Symposium on the Biological Effects of Mt. St. Helens Volcanic Ash. (1981).

10. B. Ostile. Statistics in Research, Second Edition, (Iowa State University Press, Ames, 1971) p. 370.

11. G.W. Snedecor, Statistical Methods, Sixth Edition, (Iowa State University, Press, Ames, 1971) p. 367.

12. S. Chatterjee and B. Prie. Regression Analysis by Example, (John Wiley and Sons, New York, 1977) p.

13. S.R. Searle. Linear Models, (John Wiley and Sons, New York, 1971) p.

14. Benomyl R, recommended by J.L. Robertson, Pacific Southwest Forest and Range Experiment Station, Berkeley, California.

15. C.H. Shanks, Jr. and D.L. Chase. Melandria 37, 65 (1981).

16. J.J. Brown and Y. bin Hussain, Melandria 37, 35 (1981).

17. R.C.Beckwith. Can Entomol. 102, 1475 (1970).

18. J.L.Robertson and M.I. Haverty. J. Econ. Entomol , (1980),

19. Supported by USDA Cooperative Agreement INT-80-105-CA.